# Long-term intense exposure to grass pollen can mask positive effects of allergenic immunotherapy on non-specific bronchial hyperresponsiveness

Ewa M. Świebocka[1], Piotr Siergiejko[2], Piotr Rapiejko[3], Zenon Siergiejko[4]

[1]University Children Hospital, Pediatrics, Gastroenterology and Allergology Department, Medical University of Bialystok, Bialystok, Poland
[2]University Hospital, Internal Medicine and Rheumatology Department, Medical University of Bialystok, Bialystok, Poland
[3]Military Institute of Medicine, ORL Department, Warsaw, Poland
[4]Respiratory System Diagnostic and Bronchoscopy Department, Medical University of Bialystok, Bialystok, Poland

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**Corresponding author:**
Prof. Zenon Siergiejko
Respiratory System Diagnostic and Bronchoscopy Department
Medical University of Bialystok
17 J. Waszyngtona St
15-274 Bialystok, Poland
Phone: +48 601 896 534
Fax: +48 85 664 20 02
E-mail: siergiejko@csk.pl

## Abstract

**Introduction:** There are many potential factors that can modulate bronchial reactivity, including exposure to allergens, viral infections, and medications. The aim of this study was to analyze the effect of grass pollination intensity on the bronchial reactivity in seasonal allergic rhinitis (SAR) patients subjected to subcutaneous allergenic immunotherapy (SCIT).

**Material and methods:** This study, performed between 2005 and 2008, included 41 patients with confirmed sensitivity to grass pollens and predominating symptoms of SAR, randomly assigned to desensitization by pre-seasonal or maintenance SCIT. Bronchial provocation challenge with histamine was performed before the onset of immunotherapy, and repeated three times after each pollen season covered by this study. Bronchial reactivity was analyzed with regard to grass pollination intensity in 2005–2008 (air concentration of grass pollen grains, seasonal number of days when air concentration of grass pollen reached at least 20 or 50 grains per 1 $m^3$).

**Results:** After 3 years of SCIT, a significant decrease in bronchial responsiveness was observed in the analyzed group as confirmed by an increase in $PC_{20}$ $FEV_1$ histamine values ($p$ = 0.001). An inverse tendency was observed after 2 years of SCIT, however. This second year of SCIT corresponded to the 2007 season, when a significantly higher number of days with at least 50 grains of pollen per 1 $m^3$ of air was recorded.

**Conclusions:** Fluctuations in pollination intensity observed during consecutive years of immunotherapy can influence bronchial reactivity in patients subjected to SCIT (ISRCTN Register: ISRCTN 86562422).

**Key words:** allergenic immunotherapy, bronchial provocation, bronchial reactivity, pollination monitoring, seasonal allergic rhinitis.

## Introduction

Seasonal allergic rhinitis (SAR) and allergic asthma have common pathogenesis, and their clinical manifestation is determined by the level at which the principal inflammatory process occurs [1–4]. Consequently, SAR can co-exist with bronchial hyperresponsiveness (BHR) in many cases, and many asthmatic patients suffer from rhinitis [5].

Effective methods of management, controlling both asthma and rhinitis, should inhibit BHR. This criterion is met by glucocorticoids and their combinations with long-acting $\beta_2$ agonists (LABA), as well as by anti-leukotriene agents and allergenic immunotherapy [6–8].

Subcutaneous allergenic immunotherapy (SCIT) is based on several years of subcutaneous administration of the specific allergen or allergoid at the highest well-tolerated dose. Literature evidence suggests that specific immunotherapy increases tolerance to inhalation of harmful allergens [9–11]. Also, non-specific BHR is attenuated during several years of allergenic immunotherapy [12]. However, this improvement in BHR is not as spectacular as the increase in bronchial tolerance to allergens [9, 11]. In our previous study, marked fluctuations of provocative concentration of histamine causing the 20% decrease in $FEV_1$ in comparison to baseline value ($PC_{20}$ $FEV_1$) histamine index were observed during consecutive years of allergenic immunotherapy [11]. Furthermore, other researchers reported similar findings [13].

There are many potential factors that can modulate bronchial reactivity, including exposure to allergens, viral infections, and medications [14–19]. However, a universal influence of one factor should be suspected whenever a marked shift in $PC_{20}$ $FEV_1$ values is observed in all study participants. Long-term, intense exposure to an allergen is one such possible factor.

The primary goal of our research was to compare the effectiveness of two protocols of SCIT, pre-seasonal and maintenance one, and their influence on specific and non-specific bronchial responsiveness (in press). However, marked fluctuations in bronchial reactivity noted during the course of this study stimulated us to search for the reason behind this phenomenon. We hypothesized that this variability could be associated with the intensity of grass pollination during analyzed seasons. Therefore, the aim of this ecological study was to analyze the effect of grass pollination intensity on the results of bronchial challenge with histamine performed several months after the pollination season (i.e. between November and January) in SAR patients subjected to SCIT.

**Table I.** Baseline characteristics of study participants ($n$ = 41)

| Parameter | Value |
|---|---|
| Age, mean ± SD [years] | 15.05 ±6.60 |
| Age < 18 years, *n* | 36 |
| Males, *n* | 28 |
| Height, mean ± SD [cm] | 160.90 ±17.32 |
| $FEV_1$, mean ± SD[1] | 102.15 ±10.52 |
| Median total IgE [kU/l] | 318 (244–1073)[2] |
| Median class of specific IgE against grass pollen | 5 (4–6)[2] |
| $PC_{20}FEV_1$ histamine ≤ 25 mg/ml, *n* | 21 |
| $PC_{20}FEV_1$ histamine ≤ 8 mg/ml, *n* | 9 |

*[1]of predicted value, [2]interquartile range. $FEV_1$ – forced expiratory volume in 1 s, IgE – class E immunoglobulin, $PC_{20}FEV_1$ histamine – a concentration of histamine which caused at least a 20% decrease in $FEV_1$ compared to its baseline value, SD – standard deviation*

## Material and methods

### Participants

This research was conducted between 2005 and 2008 within the framework of project number 3-18503P. All procedures were approved by the Ethics Committee of the Medical University of Bialystok (decision number R-I-003/299/2006). The subjects and/or the parents of the under-age participants gave their informed consent before the start of any procedure. Forty-one patients with sensitivity to grass pollen (as confirmed by the skin prick tests) and predominating symptoms of SAR were included in this study. Characteristics of study participants are summarized in Table I. The patients were randomly assigned to desensitization by pre-seasonal ($n$ = 20) or maintenance SCIT ($n$ = 21). Immunotherapy was based on Allergovit® 006-grass 100% preparation (Allergopharma, Germany).

### Bronchial challenge

Bronchial provocation challenge with histamine was performed before the onset of immunotherapy (between November and January), and repeated three times after each pollination season covered by this study, always at the same time of the year. The test was performed by the five-breath method in accordance with Rosenthal [20], using a DeVilbiss 646 jet nebulizer connected to a Koko Digidoser dosimeter/spirometer (Ferraris, USA), powered by compressed air. Bronchial responsiveness was quantified by the widely accepted $PC_{20}$ $FEV_1$ histamine index. The result of the test was considered positive when histamine inhaled five times at a concentration ≤ 25 mg/ml caused at least a 20% decrease in $FEV_1$ compared to its baseline value [21]. BHR, in turn, was only diagnosed when $PC_{20}$ $FEV_1$ values for histamine were below 16 mg/ml; therefore, not every positive result of the challenge corresponded to bronchial hyperresponsiveness [22]. For the purpose of statistical analysis, negative results of the provocation test were recorded as $PC_{20}$ $FEV_1$ = 50 mg/ml of histamine.

### Data on pollination

Routine independent pollination monitoring has been continued in Bialystok for several years. Data

on grass pollination intensity in 2005–2008 were kindly provided by the Allergen Research Center in Warsaw. Only the data pertaining to periods between May 1st and August 31st were analyzed.

### Experimental design

Data on the intensity of grass pollination during a given season (air concentration of grass pollen grains, seasonal number of days when the concentration of grass pollen reached at least 20 or 50 grains per 1 $m^3$ of air) were compared to the mean levels of $PC_{20}$ $FEV_1$ histamine determined at the end of the respective season. Additionally, we analyzed bronchial responsiveness of patients subjected to different protocols of SCIT.

### Statistical analysis

The normal distribution of continuous variables was tested using the Shapiro-Wilk test. The significance of time course differences among continuous variables was tested by the Friedman ANOVA test. All calculations were performed using Statistica 8 (StatSoft®, Poland) software, and statistical significance was defined as $p \leq 0.05$.

## Results

Analysis of pollination recorded in Bialystok between 2005 and 2008 revealed marked fluctuations in grass pollen concentration (Figure 1). The effects of single exposure to a high concentration of allergen have a lower impact on bronchial responsiveness compared to repeated exposure to markedly lower levels of allergen [23]. Therefore, we analyzed the number of days per season with air concentrations of grass pollen reaching one of two reference threshold levels: 1) 20 grains per 1 $m^3$ of air (when clinical symptoms of rhinitis are usually reported by most sensitive individuals), and 2) 50 grains per 1 $m^3$ (when the symptoms are reported by all sensitive individuals) [24, 25]. This analysis revealed significant differences between studied seasons: compared to other years, 2007 was characterized by a significantly higher number of days with at least 50 grains of pollen per 1 $m^3$ of air, and this parameter was the lowest in 2006 (Table II).

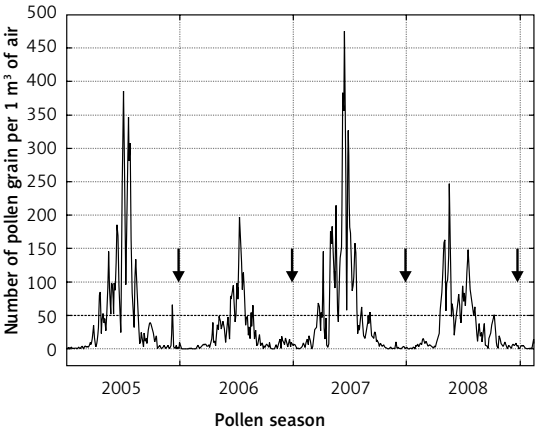


**Figure 1.** Daily concentrations of grass pollen recorded in Bialystok between 2005 and 2008. Arrows indicate the dates of bronchial challenges with histamine performed in our participants

After 3 years of SCIT, a significant decrease in bronchial responsiveness was observed in the analyzed group, as confirmed by an increase in $PC_{20}$ $FEV_1$ histamine values ($p = 0.001$; Figure 2 A). An inverse (although insignificant) tendency was observed after 2 years of immunotherapy, howev-

**Table II.** Statistical characteristics of pollination in Bialystok region in days when the concentration of grass pollen reached at least 20 or 50 grains per 1 $m^3$ of air

| Season | Days (*n*) | Number of grass pollen grains | | | |
|---|---|---|---|---|---|
| | | Mean ± SD | Median[1] | Range | Total |
| Grass pollen grains ≥ 20 per 1 $m^3$ of air | | | | | |
| 2005 | 56 | 101 ±91 | 75 (37–127) | 23–385 | 5673 |
| 2006 | 40[2] | 62 ±40 | 46 (34–79) | 21–197 | 2463 |
| 2007 | 58[3] | 107 ±99 | 68 (38–147) | 20–475 | 6216 |
| 2008 | 51 | 74 ±45 | 62 (34–94) | 21–247 | 3753 |
| Grass pollen grains ≥ 50 per 1 $m^3$ of air | | | | | |
| 2005 | 36 | 140 ±93 | 98 (77–177) | 51–385 | 5039 |
| 2006 | 17[2] | 97 ±39 | 86 (75–114) | 54–197 | 1655 |
| 2007 | 36[3] | 152 ±102 | 136 (71–175) | 54–475 | 5485 |
| 2008 | 35 | 92 ±43 | 81 (59–112) | 51–247 | 3208 |

*[1]with interquartile range; [2]significantly lower compared to 2005, 2007 and 2008 ($p < 0.05$); [3]significantly higher than in 2006 ($p < 0.05$). SD – standard deviation*

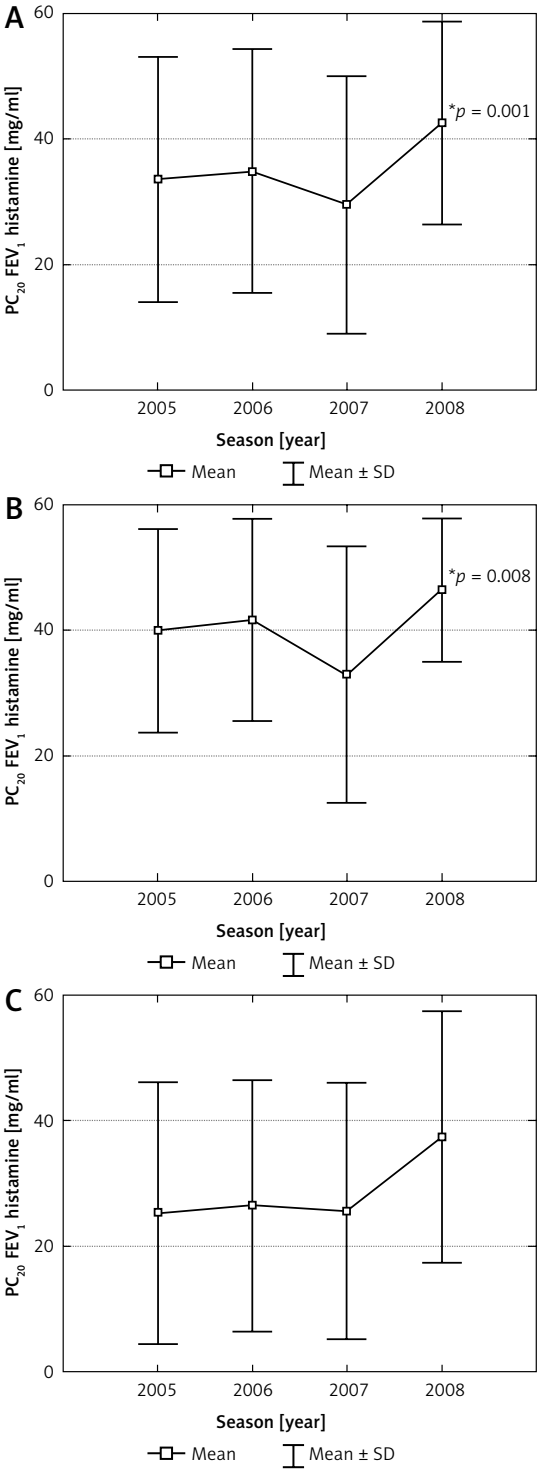


**Figure 2.** Mean (± SD) levels of $PC_{20}$ $FEV_1$ histamine in studied subjects determined during consecutive seasons of SCIT: **A –** entire group ($n$ = 41), **B –** patients subjected to maintenance SCIT ($n$ = 21), **C –** patients subjected to pre-seasonal SCIT ($n$ = 20)

er, when a decrease in $PC_{20}$ $FEV_1$ histamine was noted. Noticeably, this second year of SCIT corresponded to the 2007 season, when a significantly higher number of days with at least 50 grains of pollen per 1 $m^3$ of air was recorded.

Additionally, a decrease in $PC_{20}$ $FEV_1$ histamine was observed after the second year of SCIT both in patients subjected to the maintenance immunotherapy (Figure 2 B) and in those qualified for pre-seasonal SCIT (Figure 2 C).

## Discussion

This study revealed that the significant reduction of non-specific bronchial reactivity is observed not earlier than after 3 years of allergenic immunotherapy. The difference between baseline and final (determined after 3 years of SCIT) bronchial reactivity was evident in the entire studied group ($n$ = 41) as well as in individuals subjected to different protocols of SCIT.

According to the literature, not every case needs as long as 3 years to reveal positive effects of SCIT on bronchial reactivity in SAR patients [12]. On the other hand, other authors observed no significant decrease in BHR during SCIT [26]. Moreover, the results of our study suggest that extremely intense natural allergen stimulation occurring in some years can markedly hinder the evaluation of SCIT effects on bronchial reactivity.

Our assumption that the intensity of pollination during the season preceding bronchial challenge with histamine can modulate the result of this test seems reasonable in view of the literature evidence. Several days of allergen stimulation of the bronchi occurring during the pollen season enhance minimal persistent bronchial inflammation in SAR patients and can modulate the degree of their bronchial reactivity [27–29]. Undoubtedly, BHR could be increased due to yearly exposure to the allergen. In one previous study, long-term avoidance of contact with an allergen was reflected in a reduced BHR [30].

The studies where inhalatory glucocorticoids were used in asthma management confirmed that once acquired, BHR usually persists for many months [31–33]. Application of inhalatory glucocorticoids is reflected by a relatively fast clinical improvement manifested by lower severity of clinical symptoms and decreased consumption of rescue medications. However, reduction in bronchial responsiveness is markedly less pronounced [33]. According to Haahtela *et al.* [34], 2 years are required for bronchial reactivity normalization in mild asthma patients who received inhalatory glucocorticoids. One may assume that despite lower intensity, the bronchial reactivity of SAR patients is similar to that observed in subjects suffering from mild asthma. The duration of allergen stimulation is relatively short in patients sensitive to grass pollen, and therefore seasonal fluctuations in bronchial reactivity can be observed [35–37].

The results of our study suggest that BHR is still observed several months after the pollination

season during the years when air concentrations of grass pollen were higher. This phenomenon significantly hindered evaluation of the specific immunotherapy effects on bronchial reactivity.

Some authors did not observe positive changes in bronchial reactivity of SAR patients who were subjected to specific immunotherapy [26]. In this study, a tendency towards reduced bronchial responsiveness was observed as early as 1 year after SCIT. After the second year, however, an inverse tendency was observed or at least improvement was no longer visible. Noticeably, the first year of this experiment (i.e. 2006) was characterized by the least intense grass pollination in our region when compared to other seasons covered by this study, whereas the intensity of pollination was the highest in 2007. Although direct comparing of pollination intensity figures to $PC_{20}$ $FEV_1$ histamine values determined after the respective season is evidently a simplification, a decrease in this latter parameter observed after the second year of SCIT corresponded to the 2007 season, when a significantly higher number of days with at least 50 grains of pollen per 1 $m^3$ of air was recorded. One can point out potential limitations of this study, namely the small number of participants and ecological character. We are well aware of these issues; nonetheless, our findings substantiate further research on the influence of pollination intensity on the effects of allergenic immunotherapy.

In conclusion, fluctuations in pollination intensity observed during consecutive years of immunotherapy can markedly hinder the evaluation of SCIT effects on bronchial reactivity.

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